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# Regulation of adhesion molecule expression in human endothelial and smooth muscle cells by omega-3 fatty acids and conjugated linoleic acids: Involvement of the transcription factor NF-κB?

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**Abstract**

We previously showed conjugated linoleic acids (CLA) inhibited TNF-α-induced monocyte (THP-1) adhesion to human umbilical vein endothelial cells (HUVEC) *in vitro* which involved an increase in platelet activating factor (PAF). Here we show adhesion molecule (ADM) regulation by fatty acids and the differing role of nuclear factor kappa B (NF-κB) activation in HUVEC and vascular smooth muscle cells (vSMC). CLA and omega-3 long-chain polyunsaturated fatty acids (PUFA) (FA) reduced TNF-ά-induced expression of ADMs (intercellular adhesion molecule-1 (ICAM-1); vascular cell adhesion molecule-1 (VCAM-1) but not E-selectin) on HUVEC and vSMC to different extents depending on FA type and concentration, cell type and method of analysis. IκBά phosphorylation in HUVEC and vSMC and transient transfection with NF-κB-luciferase reporter plasmid (HUVEC only) indicated differential NF-κB involvement during FA modulation (*cis*-9, *trans*-11; *trans*-10, *cis*-12 and a 50:50 mix of both CLA isomers; eicosapentaenoic acid (EPA); docosahexaenoic acid (DHA)). TNF-α-induced ADM expression in both cell types by 2–10-fold. In HUVEC, CLA *t*10, *c*12 and CLA mix (50:50 mixture of CLA *c*9, *t*11 and *t*10, *c*12) and EPA and DHA reduced ICAM-1 expression (15–35%) at 12.5, 25 and/or 50 μM. VCAM-1 expression was reduced by 25 μM *t*10, *c*12 isomer and mix; omega-3 PUFA and other concentrations of CLA and TNF-α-induced E-selectin expression were unaffected. TNFα-induced inhibitor kappa B (IκB) phosphorylation was biphasic peaking at 5 min in both cell types and 60 and 120 min in HUVEC and SMC, respectively. IκBα phosphorylation and NF-κB activity was reduced (29% and 30%, respectively) by 25 μM CLA mix. n-3 PUFA did not reduce IκBα phosphorylation or NF-κB activity but reduced ADM expression. We show that n-3 PUFA and CLA reduce expression of ADM on HUVEC and vSMC. This reflected reduced adherence of monocytes to HUVEC previously reported by our group. Reduction of ICAM-1 and VCAM-1 protein expression by n-3 PUFA was less dependent on the NF-κB pathway than reduction by CLA which reflected the parallel attenuation of NF-κB activity. This indicated involvement of other transcription factors (i.e. AP-1) in the FA regulation of ADM expression and has, to our knowledge, not been previously reported.


## 1. Introduction

Atherosclerosis, a disease of the large arteries, is a major cause of death in the Western world, being the underlying process leading to heart disease and stroke [1,2]. Adhesion molecules (ADMs) are responsible for cell–cell interactions including the "rolling" of the monocytes/leukocytes along the endothelium as well as their adherence and extravasation. Intercellular

*Abbreviations:* AA, arachidonic acid; DHA, docosahexaenoic acid; CLA, conjugated linoleic acids; EC, endothelial cells; EPA, eicosapentaenoic acid; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; IκB, inhibitor kappa B; IKK, inhibitor kappa B kinase; LA, linoleic acid; NF-κB, nuclear factor kappa B; LC-PUFA, long-chain polyunsaturated fatty acids; vSMC, vascular smooth muscle cells; VCAM-1, vascular cell adhesion molecule-1.

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adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are members of the immunoglobulin super-family and are both expressed in endothelial cells (EC). They are increased under pro-oxidative and pro-inflammatory stimuli and subsequently promote the adhesion of leukocytes as well as their diapedesis through the endothelium into the intima of the vessel wall [1–3].

It has been reported that treatment with n-3 long-chain, polyunsaturated fatty acids (n-3 LC-PUFA) such as eicosapentaenoic acid (EPA-20:5n-3) and docosahexaenoic acid (DHA-22:6n-3) present in fish oils, decreased ICAM-1 and VCAM-1 mRNA and protein on TNF-α-stimulated EC [3–5] and stabilized the atherosclerotic plaque [6]. Other types of fatty acids, namely conjugated linoleic acids (CLA), have also been implicated in the prevention and regression of atherogenesis in animal models of the disease [7]. Furthermore, CLA, obtained from ruminant meat and dairy products, also have beneficial effects on tumor progression in cancer and they can prevent metastasis in animal models of disease [8]. This is likely due to circulating cancer cells utilizing the same ADMs for tissue invasion as the monocytes in intimal plaque formation and consequently a reduction in their expression would also result in reduced metastatic potential [9].

ADM expression in human umbilical vein endothelial cells (HUVEC) is regulated by different transcription factors such as nuclear factor kappa B (NF-κB), AP-1 and peroxisome proliferator-activated receptors (PPAR) [10,11]. The ICAM-1 gene promoter possesses one response element for NF-κB whilst the VCAM-1 promoter contains two and that of E-selectin contains three [12]. When cytosolic NF-κB is activated in response to inflammatory and/or oxidative stimuli, it translocates to the nucleus, binds to specific gene promoters involved in stress responses and triggers the transcription of these stress-response genes, which includes the up-regulation of ADMs, cytokines and heat shock proteins. In non-stimulated cells, NF-κB is bound to inhibitory-inhibitor kappa B (IκB) in an inactive complex in the cytoplasm [13]. The phosphorylation of IκB by specific inhibitor kappa B kinase (IKK) (IκB kinase), followed by polyubiquitination and proteosomal degradation releases free NF-κB, which translocates to the nucleus and binds to κB sites in specific gene promoters thereby stimulating their transcription. Consequently this phosphorylation step is regarded as the initiating step in the NF-κB stress cascade [13].

Fatty acids can influence the NF-κB signaling pathway. Camandola et al. [14] showed that arachidonic acid (AA) strongly up-regulated NF-κB nuclear translocation (activation) in U937 monocytes but EPA was without effect. Moreover, recent data from our group demonstrated that CLA elicited a reduction in cytokine-induced NF-κB activation in prostate cancer cells by reducing IκBα phosphorylation (activation) as well as the translocation of NF-κB to the nucleus [15].

The objectives of the current study were, firstly, to compare the effects of CLA and n-3 PUFA on TNF-α-induced ICAM-1, VCAM-1 and E-selectin protein expression, in HUVEC and SMC using Western blot and/or flow cytometric analysis, and then to determine if these effects support our previously published findings that these fatty acids inhibit ADM mRNA expression and monocyte binding in HUVEC *in vitro* [3–5,16–18]. Secondly, it was to determine the effect of these fatty acids on the regulation of the NF-κB transcription pathway that is believed to be responsible, at least in part, for ADM expression in these cells, by determining their effects on IκBα phosphorylation and on the NF-κB-luciferase reporter activity in HUVEC. Our findings show that CLA, similar to the n-3 PUFA, can down-regulate TNF-α-induced ICAM-1 and VCAM-1 ADM expression in HUVEC and vascular smooth muscle cells (vSMC) and that this correlates with the attenuation of the actual binding of monocytes on the TNF-α-stimulated HUVEC as we reported previously. Our findings also indicate that transcription factors other than NF-κB appear to be involved in ADM regulation depending on the type of fatty acid present, e.g. CLA or omega-3 PUFA. To our knowledge such differences in effects of specific fatty acids on ADM signaling pathways in HUVEC have not been previously reported.

## 2. Materials and methods

### 2.1. Reagents

Heparin, endothelium growth factor supplement (ECGS), collagenase, TNF-α, protease inhibitor cocktail and linoleic acid (LA) were supplied by Sigma (Poole, Dorset, UK). Medium 199, PBS, penicillin/streptomycin were purchased from GibcoBRL (Paisley, UK). Fetal calf serum (FCS) was purchased from Perbio (Tattenhall, UK). The fatty acids EPA, DHA and palmitic acid (PA) were supplied by Pronova (Sandefjord, Norway) and CLA (CLA *cis*-9, *trans*-11 (CLA *c*9, *t*11), CLA *trans*-10, *cis*-12 (CLA *t*10, *c*12) and CLA mix [(50:50) CLA *c*9, *t*11, CLA *t*10 *c*12]), for a concentration of 25 μM CLA mix, there is 12.5 μM CLA *c*9, *t*10 and 12.5 μM CLA *t*10, *c*12] by Natural ASA (Hovdebygda, Norway). Bis-acrylamide and BioRad Dc protein assay kit, reagents A and B, were purchased from BioRad (Hemel Hempstead, UK). PVDF membrane was obtained from Amersham Pharmacia (Little Chalfont, UK). Anti-ICAM-1, anti-VCAM-1, anti-E-selectin and anti-rabbit antibodies were purchased from Santa Cruz (Autogen Bioclear, Calne, UK); anti-phosphorylated IκBα antibody from Cell Signaling (New England